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(57) Abstract

The present invention provides a novel method for identifying individuals who are likely to have negative responses to regular administration of β–agonists. The invention also provides kits useful for this purpose.

DIAGNOSING ASTHMA PATIENTS PREDISPOSED TO ADVERSE β-AGONIST REACTIONS

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Background of the Invention

Inhaled medium acting β-agonists are the most commonly prescribed asthma treatments in the world. β-agonists produce their effects by stimulating the β_2-adrenergic receptors on cells and thereby activating intracellular pathways that produce increased levels of cyclic adenosine monophosphate (cAMP). The increased intracellular cAMP levels in turn produce macroscopic effects in the cells, relaxing the smooth muscles of the bronchial airways, increasing ciliary beat frequency, and reducing mucous viscosity. The effectiveness of β-agonists at dilating bronchial airways has led to their widespread administration both as a treatment for acute asthmatic episodes and as a long-term asthma management therapy.

Concerns about the safety of β-agonist therapy have arisen periodically over the years (reviewed in, for example, Taylor et al., *Med. Clin. N. America* 80:719, 1996; Giunti et al., *Eur. Respir. J.* 8:673, 1995; Barrett et al., *Am. J. Respir. Crit. Care Med.* 151:574, 1995; Devoy et al., *Chest* 107:1116, 1995; McFadden, *Ann. Allergy Asthma*

Immunol. 75:173, 1995; Crane et al., *Thorax* 50:S5, 1995; McFadden, *J. Allergy Clin. Immunol.* 95:41, 1995). Reports of possible associations between β-agonist administration and increased morbidity, particularly for chronic β-agonist administration protocols, have spurred much debate over the safety of β-agonist therapy. There is a need to resolve this debate and to identify risks of deleterious or salutory effects associated with administration of β-agonsists to asthmatics.

Summary of the Invention

The present invention resolves the debate over the safety of β-agonist therapy and identifies a population of asthmatic patients who are at risk for an adverse reaction to regular administration of β-agonists. In particular, the present invention provides the discovery that asthmatics who carry a particular allele of the β_2-adrenergic receptor gene are more likely to have a negative response to chronic β-agonist therapy. The present invention provides methods of identifying individuals at risk for an adverse response to β-agonist treatment, and also provides diagnostic kits useful in the practice of such methods.

In preferred embodiments of the methods of the present invention, a genomic nucleic acid sample is provided from an individual, first and second β_2-adrenergic receptor gene alleles are identified within the genomic nucleic acid sample, and any individual for whom both the first and second β_2-adrenergic receptor gene alleles encode Arg at residue 16 is classified as being at risk for adverse reaction to chronic β-agonist administration. The particular method by which the β_2-adrenergic receptor gene alleles are identified within the genomic sample is not intended to limit the scope of the present

invention. However, preferred identification methods include allele-specific polymerase chain reaction (PCR) techniques and direct sequencing techniques.

Preferred kits provided by the practice of the present invention include reagents useful for performing the inventive methods, which reagents are assembled together in a container for ease of use.

Description of the Drawings

Figure 1 depicts the primary amino acid sequence and known polymorphic sites in the human β_2-adrenergic receptor protein. Nine polymorphic sites are shown; those shown in black represent different gene alleles that encode the same residue, whereas those in white that are labeled with alternate amino acids represent gene alleles that result in residue substitutions.

Figure 2 is a photograph of an agarose gel presenting genotype analyses of six BAGS patients at residue 16 of the β_2 adrenergic receptor.

Figure 3 has panels A and B, showing the morning and afternoon peak expiratory flow rates (PEFR), respectively, of asthmatics who received regularly- scheduled or as-needed albuterol treatments. In each panel, the results are plotted by β_2-adrenergic receptor genotype. Data from asthmatics who are homozygous for the β_2-adrenergic receptor Arg16 variant are plotted either as a solid line punctuated with diamonds (those who received regular treatment) or a gray line punctuated by squares (those who received as-needed treatment); data from Gly16 homozygotes who received regular treatment are plotted as a light gray line punctuated by triangles; data from Arg16/Gly16 heterozygotes are plotted as light gray line punctuated by Xs.

Description of the Sequences

SEQ ID NO:1 presents an amino acid sequence of the β_2-adrenergic receptor. In SEQ ID NO:1, Arg, Gln, Val, and Thr are located at positions 16, 27, 34, and 164, respectively. Known gene polymorphisms produce β_2-adrenergic receptors with Gly, Glu, Met, and Ile, respectively, at these positions (see Figure 1).

SEQ ID NO:2 presents a human β_2-adrenergic receptor gene encoding the protein of SEQ ID NO:1. The Arg16 → Gly polymorphism described above with respect to SEQ ID NO:1 can be produced by substituting a G for the A at SEQ ID NO:2 position 1633; the Gln 27 → Glu polymorphism can be produced by substituting a G for the C at 1666.

Description of Preferred Embodiments

The human gene for the β_2-adrenergic receptor has been cloned (Kobilka et al., *Proc. Natl. Acad. Sci. USA* 84:46, 1987) and extensively studied. Nine gene polymorphisms have been identified in the general population, four of which result in amino acid substitutions (the other five are silent changes) (see Figure 1; see also, Reihsaus et al., *Am. J. Respir. Cell. Mol. Biol.* 8:334, 1993). The present invention relates to the "Arg16 → Gly" polymorphism depicted in Figure 1.

Various studies have been undertaken to identify any significance of the Arg16 → Gly polymorphism in asthma (for review, see Liggett, Chapter 21, *The Genetics of Asthma* [Liggett et al., eds], Marcel Dekker, NY, 1996). No general association between either the Arg16 or the Gly16 allele and asthma has been observed (Reihsaus et al., *Am. J. Respir. Cell. Mol. Biol.* 8:334, 1993). Also, the Arg16 and the Gly16

proteins have been shown to have equivalent affinities for agonists and antagonists, and to couple normally to G_s (Green et al., *Biochemistry* 33:9414, 1994).

The only difference observed prior to the present invention between the Arg16 and Gly16 allele was enhanced down regulation of the Gly16 allele in response to β-agonist administration (Green et al., *Biochemistry* 33:9414, 1994). This finding prompted one expert in the field to conclude that:

> "the Gly16 variant, which undergoes the greatest degree of agonist promoted down regulation, would be expected to display an overall reduced level of expression as compared with [the Arg16 variant]. Under this scenario, basal bronchomotor tone might be decreased or bronchial hyperactivity increased. . . . Responsiveness to β-agonist may also be depressed in asthmatics harboring [the Gly16] polymorphism. . . . During chronic agonist therapy, the potential for tachyphylaxis would appear to be greatest with the Gly16 variant. . . . Given reports that suggest a relationship between 'overuse' of β-agonists and adverse outcomes in asthma, it seems prudent to consider that tachyphylaxis may occur in some individuals"

(citations omitted; Ligget, Chapter 21, *The Genetics of Asthma* (Ligget et al., eds), Marcel Dekker, NY, 1996, pg. 470). Thus, prior to the present invention, the state of knowledge concerning the Arg16 $\rightarrow$ Gly polymorphism in the human β_2-adrenergic receptor indicated that individuals carrying the Gly16 allele might suffer more tachyphylaxis in response to chronic β-agonist therapy, and therefore might be more susceptible to adverse responses to β-agonist administration.

Surprisingly, the present invention provides the discovery that the opposite is true: individuals carrying the Gly16 allele of the human β_2-adrenergic receptor are actually *less* susceptible to adverse responses to β-agonist administration. According to the present invention, individuals who are homozygous for the Arg16 allele are more likely to have an adverse response to β-agonist therapy than either Gly16 homozygotes or Gly16/Arg16 heterozygotes.

As described in Example 2, we analyzed the β_2-adrenergic receptor genotype in 179 subjects who had participated in a study testing their response to regular and as-needed albuterol administration (Drazen et al., *New Eng. J. Med.* 335:841, 1996, incorporated herein by reference; see also Example 1). That study had concluded that "in patients with mild asthma, neither deleterious nor beneficial effects derived from the regular use of inhaled albuterol beyond those derived from the regular use of the drug as needed (Drazen et al., *id.*). When we examined the β_2-adrenergic receptor genotype in the study participants, however, we came to a rather different conclusion. Figure 2 shows our findings: individuals who carry two copies of the Arg16 β_2-adrenergic receptor allele showed significant decreases in peak expiratory flow rate (PEFR) after receiving regular β-agonist therapy for 16 weeks. Gly16 homozygotes and Arg16/Gly16 heterozygotes did not show this effect. Arg16 homozygotes who received β-agonist treatments on an as-needed basis showed more modest decreases in PEFR and these decreases were only temporary.

By demonstrating a correlation between adverse response to chronic β-agonist therapy and the presence of (two copies of) the Arg16 β_2-adrenergic receptor allele, the present invention provides methods for identifying asthmatic patients at risk of such an

adverse response. Quite simply, patients are screened to identify the β_2-adrenergic receptor alleles they carry; those who are homozygous for Arg16 are identified as susceptible. Any available method can be used to detect patients' β_2-adrenergic receptor genotype (see, for example, Examples 2 and 3; see also methods described in *Current Protocols in Human Genetics*, John Wiley & Sons, Unit 9, incorporated herein by reference).

For example, the relevant region of each patients' β_2-adrenergic receptor gene (both alleles) can be directly sequenced according to known techniques. Alternatively or additionally, techniques such as denaturing gradient gel electrophoresis, allele-specific polymerase chain reaction (PCR), allele-specific hybridization, allele-specific ligation amplification (see, for example, English et al., *Proc. Natl. Acad. Sci. USA* 9:360, 1994, incorporated herein by reference), single strand conformation polymorphism analysis, restriction fragment length polymorphism analysis, or any other available technique useful to distinguish sequence polymorphisms may be employed. Allele-specific PCR techniques, such as the amplification refractory mutation system (ARMS), or amplification followed by sequencing, are preferred methods of polymorphism detection. Preferred hybridization methods include hybridization to oligonucleotides on a silica chip array (see, for example, Hacia et al, *Nature Genetics* 14:441, 1996, incorporated herein by reference; see also *Nature Genetics* 14:367, 1996).

The present invention also provides kits for identifying asthma patients susceptible to adverse responses to chronic β-agonist administration. Preferred kits comprise reaction components useful for allele-specific PCR techniques. For example, particularly preferred kits include primer sets capable of amplifying and distinguishing the Arg16 and

Gly16 alleles, and may also include buffers, thermalstable reverse transcriptase, control templates, etc. Alternative preferred kits include amplification reagents that are not necessarily allele-specific, in combination with sequencing reagents.

Examples

EXAMPLE 1

Analysis of Beneficial and Deleterious Effects of Regularly-Scheduled and As-Needed Albuterol Administration in Patients with Mild Asthma

(see Drazen et al., *NEJM* 335:841, 1996, incorporated herein by reference)

Materials and Methods

PATIENT RECRUITMENT: Patients with mild asthma, as defined by the criteria shown in Table 1, were recruited from existing study populations and by advertising. Eligible patients entered a six-week single-blind run-in period, during which they used a placebo inhaler on a regular basis (two inhalations four times a day) and took supplemental puffs of open-label albuterol as needed. During the run-in period, patients were evaluated three times at two-week intervals, at which time asthma control was assessed by the review of a number of criteria.

TABLE 1
CHARACTERISTICS USED TO DEFINE MILD ASTHMA*

CHARACTERISTIC	ALLOWABLE RANGE
FEV_1†	≥70% of predicted value
Age	12 to 55 yr
PC_{20}	≤ 16 mg/ml
Use of β-agonists	6 to 56 puffs of albuterol/wk; patients using less than 6 puffs of albuterol/wk at visit 1 had to have a PC_{20} of ≤ 8 mg/ml

Use f other asthma medications	N ne, no corticosteroids for 6 wk
Other serious medical conditions, including pregnancy	Not allowed
Smoking	None in past year, maximal history of 5 pack-years permitted

*FEV_1 denotes forced expiratory volume in one second, and PC_{20}, the concentration of methacholine required to decrease the FEV_1 by 20 percent.
†The FEV_1 was measured after at least eight hours without bronchodilator medications.

PATIENT SELECTION: Patients were randomly assigned to a treatment group if over the six-week period their asthma was clinically stable and they demonstrated their ability to comply with the study procedures, as indicated by the regular use of the placebo inhaler (monitored by a Chronology recording device) and their ability to record their peak flow (twice daily, using a Mini-Wright peak-flow meter) (Clement Clarke, Columbus, OH) and asthma symptoms once daily in a diary. The treatments assigned consisted of either inhaled albuterol on a regular basis (two inhalations four times a day) plus albuterol as needed or inhaled placebo on a regular basis (two inhalations four times a day) plus albuterol as needed. Albuterol and placebo inhalers were generously supplied by Schering-Plough (Memphis, TN). Patients were instructed to have their regularly scheduled inhalations in the morning after recording their morning peak flow, at midday, in the late afternoon, and on retiring to sleep after recording their evening peak flow. They were instructed to allow at least four hours between their regularly scheduled inhalation in the late afternoon and the recording of their evening peak flow.

PATIENT TREATMENT: Over the ensuing 16 weeks, while patients received blinded treatment, the control of asthma was monitored daily, through peak flow rates and symptoms recorded by patients, as well as during clinic visits, which were scheduled every two to three weeks. At the completion of the randomized-treatment period, all the

patients were switched to single-blind treatment with inhaled placebo for a four-week withdrawal period; during this time patients continued to use open-label albuterol as needed.

Seven outcome indicators were monitored: peak flow, the symptom record, quality of life, the change in the forced expiratory volume in one second (FEV_1) in response to an inhaled bronchodilators, the concentration of methacholine required to decrease the FEV_1 by 20 percent (PC_{20}), asthma exacerbations, and treatment failure. Peak flow, the primary outcome indicator, was measured twice daily by patients using a Mini-Wright peak-flow meter; the best of three efforts was recorded. Patients recorded their asthma symptoms and the number of puffs of supplemental albuterol used daily. Asthma symptoms were recorded on a 4-point scale, with 0 representing no symptoms and 3 representing severe symptoms. Asthma-specific quality-of-life scores were recorded during clinic visits, with an instrument validated by other investigators (Juniper et al., *Thorax* 47:76, 1992). To determine the spirometric response to an inhaled bronchodilator, the difference in the FEV_1 before and 15 minutes after two inhalations of albuterol was measured (and reported as percent improvement) during clinic visits when responsiveness to methacholine was not tested.

Patients refrained from taking their study medications for at least eight hours before all clinic visits. To measure PC_{20} for methacholine, methacholine aerosols were generated with a nebulizer (model 646, DeVilbiss Health Care, Somerset, Pa.) and a calibrated dosimeter (S&M Instruments, Dovestown, PA). The PC_{20} for methacholine was determined by standard procedures (Tashkin et al., *Am. Rev. Respir. Dis.* 145:301, 1992). Asthma exacerbations were monitored during each clinic visit; patients were

asked about their asthma control, and all asthma exacerbations were recorded. An asthma exacerbation was defined as an increase in symptoms of cough, chest tightness, or wheezing in association with one or more of the following; an increase over the base-line use of supplemental β-agonist treatments of 8 or more puffs per 24 hours for a period of 48 hours, the use of 16 or more puffs of a supplemental β-agonist per 24 hours for a period of 48 hours, or a fall in peak flow of 35 percent or more from the best three-day average (morning and evening) during the run-in period.

Treatment was considered to have failed if patients who had asthma exacerbations and were treated with increased doses of β-agonists did not respond adequately — that is, if they continued to meet the criteria for exacerbation. Such patients were treated with a short course of prednisone, as determined by their physicians; their data continued to be collected, and they remained in the trial (in accordance with the intention-to-treat method).

STANDARDIZATION AND QUALITY-ASSURANCE TECHNIQUES: All clinical laboratory tests — that is, measurements of lung function, skin testing for allergies, methacholine challenges, and quality-of-life assessments — were performed at each center with the use of equipment and procedures that were standardized for the entire network. Workers participating in the network were tested to ensure proficiency and uniformity in all network-related skills and had to pass certification examinations before the data they gathered could be used in the network. All results of spirometric testing (Collins Eagle 2 spirometer, Quincy, MA), including that for the methacholine challenge, were confirmed by a single network member. Peak-flow meters were tested against spirometers during each clinic visit and were replaced if they failed to meet previously established

performance standards. A distributed data-entry system allowed each clinical center to submit its data over the Internet directly to the Data Coordinating Center. The Data Coordinating Center entered the data a second time to verify it.

COMPLIANCE: Each patient was given a digital wristwatch with multiple alarms to improve treatment compliance. In addition, Chronology recording devices were used with the randomly assigned metered-dose inhalers to provide an electronic record of the date and time of inhaler use.

STATISTICAL ANALYSIS: Morning peak flow was chosen as the primary outcome variable for the calculation of sample size. A Minimum of 200 patients made it possible to detect a difference of 25 liters per minute between groups with 80 percent statistical power. A goal of recruiting 250 randomized patients was established on the assumption that the dropout rate would be less than 20 percent. This sample size also provided 80 percent statistical power to detect a difference of 0.19 liter in FEV_1 and 0.70 doubling dilution in the PC_{20} values for methacholine.

Response variables — that is, peak-flow values, medication use, and asthma symptoms — from the patients' diary cards were averaged each week. Because of the longitudinal nature of most of the response variables, a mixed-effects linear model was applied (Vonesh et al., *Biometrics* 43:617, 1987; Laird et al., *Stat Methods Med Res* 1:225, 1992); this approach allowed all data obtained to be used, not just the data obtained at a single visit. For each response variable, a segmented linear model was fitted with an intercept and with slopes for the last 4 weeks of the run-in period, the first 5 weeks of the treatment period, the remaining 11 weeks of the treatment period, and the withdrawal period. The "break point" after five weeks of randomized treatment was

chosen on the basis of rates of asthma exacerbation reported by Sears et al. (Sears et al., *Lancet* 336:1391, 1990).

For each outcome measure, values were calculated from the models for the end of the run-in period, for the end of the double-blind-treatment period, and for the end of the withdrawal period. This statistical model was determined before the start of the study, and therefore other models were not considered during data analysis. The groups were compared with respect to rates of treatment failure with the use of Fisher's exact test. To ensure patient safety, an interim analysis was conducted after approximately 40 percent of the randomized patients had completed the trial or withdrawn consent; as a result of this analysis, the P value considered to indicate statistical significance was reduced from 0.05 to 0.03 for the final analyses (Pocock, *Biometrics* 38:153, 1982; Geller et al., *Biometrics* 43:213, 1987).

Results

ENROLLMENT AND RETENTION: Of the subjects recruited, 255 were eligible for enrollment at the end of the six-week run-in period and were randomly assigned to receive double-blind treatment (Table 2). There were no significant differences between the treatment groups with respect to any of the indexes monitored. During the period of randomized treatment and withdrawal, 25 subjects dropped out of the trial — 10 in the scheduled-treatment group and 15 in the treatment-as-needed group. Two hundred thirty patients completed the entire trial.

TABLE 2
CHARACTERISTICS OF PATIENTS IN THE TWO TREATMENT GROUPS

CHARACTERISTIC*	ALBUTEROL TREATMENT†	
	REGULARLY SCHEDULED (n=126)	AS-NEEDED ONLY (N=129)
Male sex — no. (%)	57 (45.2)	55 (42.6)
Minority racial or ethnic group — no. (%)‡	41 (32.5)	43 (33.3)
Atophy — no. (%)	122 (96.8)	127 (98.4)
Age — yr	28.6±9.0	29.3±9.2
Age <18 yr — no. (%)	16 (12.7)	10 (7.8)
Morning peak flow — liters/min§	418.3±100.5	421.6±99.8
Evening peak flow — liters/min§	437.6±101.5	440.7±99.1
Peak-flow variability — %§¶	3.9±5.7	3.7±6.9
Symptom score§‖	0.46±0.40	0.38±0.34
No. of supplemental puffs of β-agonist per day§	1.5±2.0	1.7±2.2
FEV_1 — liters¶ (% of predicted value)**	3.1±0.74 (89.0±12.7)	3.15±0.68 (91.4±13.9)
Quality-of-life score**††	2.28±0.82	2.44±0.82
PC_{20} — mg/m**‡‡	0.64±1.82	0.64±1.82
FEV_1 response to albuterol inhalation — % change from base line §§	10.5±8.3	10.8±9.2

*FEV_1 denotes forced expiratory volume in one second, and PC_{20} the concentration of methacholine required to decrease the FEV_1 by 20 percent.
†Plus-minus values are means ±SD unless otherwise indicated.
‡Fifty-nine percent of the minority patients in the scheduled group were black, and 65 percent in the as-needed group were black.
§Values represent averages for the last four weeks of the run-in period.
¶Peak-flow variability was calculated as ([evening peak flow — morning peak flow] ÷ evening peak flow) x 100.
‖Asthma symptoms were graded by the patient each day, from 0 for no symptoms to 3 for incapacitating symptoms.
**This characteristic was measured from week 6 of the run-in period.
††Asthma-specific quality-of-life questionnaires were completed by the patients during clinical-center visits. A score of 1.0 indicates that asthma had no effect on the overall quality of life; a score of 2.0, that the patient's life was "a little limited" by asthma; a score of 3.0, that there was "some limitation"; and a score of 7.0, that there was "total limitation."
‡‡Values are medians and interquartile ranges.
§§Data are the averages from weeks 2 and 4 of the run-in period.

COMPLIANCE: Compliance with the use of inhaled medication, either active or placebo, on a regular basis was greater than 80 percent, as indicated by Chronolog treatment records and an analysis of diary cards. Of the 3172 scheduled visits to patients' clinical center, 26 were missed, for a rate of compliance of over 99 percent.

ASTHMA EXACERBATIONS: Asthma was exacerbated 24 times (11 times in the scheduled-treatment group and 13 times in the treatment-as-needed group) during the active treatment period and 4 times during the withdrawal period (twice in each treatment group). The 28 exacerbations occurred in 12 patients in the scheduled-treatment group and 11 patients in the treatment-as-needed group.

TREATMENT FAILURES: Treatment was considered to have failed in 11 patients during the 16-week period of randomized treatment (5 in the scheduled-treatment group and 6 in the treatment-as-needed group) and in 2 during the withdrawal period (both in the scheduled-treatment group). There were three visits to the emergency room for asthma (two in the scheduled-treatment group and one in the treatment-as-needed group). No patients were hospitalized for asthma during the trial, and none died. There were no significant differences in any of the event rates between the two treatment groups.

EFFICACY OUTCOMES: Lung function (indicated by morning peak flow, evening peak flow, peak-flow variability, FEV_1, β-agonist responsiveness, and PC_{20}) and asthma symptoms (determined by the number of uses of the supplemental β-agonists metered-dose inhalers, diary scores and quality-of-life scores) as derived from the regression analysis performed for each patient group are shown in Table 3. Graphic displays of values predicted by the model as compared with sample means showed excellent goodness of fit by the statistical model (data not shown). There were no significant differences in

morning peak flow between the two treatment groups (Table 3). Even though the average use of albuterol was 9.3 puffs per day in the scheduled-use group and 1.6 puffs per day in the treatment-as-needed group, the extra use of medication did not lead to differences in peak-flow variability, FEV_1, supplemental albuterol use, asthma symptoms, quality of life, or PC_{20}.

Two significant differences were found between the groups. Once was in the change in evening peak flow from the end of the treatment period to the end of the withdrawal period; mean evening peak flow fell 17.7 liters per minute in the scheduled-treatment group but increased 1.3 liters per minute in the treatment-as-needed group. The other significant difference was in the change in bronchodilator responsiveness between the run-in period and the treatment period (Table 3). The FEV_1 response to treatment with albuterol increased from a 10.7 percent improvement to a 12.5 percent improvement in the scheduled-treatment group and decreased from a 10.7 percent improvement to a 9.2 improvement in the treatment-as-needed group. A number of small but statistically significant changes within the groups were noted among the various treatment periods, as shown in Table 3. Results of the analysis in which data collected after the subjects in whom treatment was considered to have failed were excluded were essentially the same as those derived with the use of the intention-to-treat method.

TABLE 3
MODEL ESTIMATES (USING INTENTION-TO-TREAT DATA) FOR THE END OF THE RUN-IN PERIOD (WEEK 6), THE END OF THE ACTIVE-TREATMENT PERIOD (WEEKS 22), AND THE END OF THE WITHDRAWAL PERIOD (WEEK 26).*

OUTCOME†	AFTER RUN-IN PERIOD		AFTER TREATMENT PERIOD		AFTER WITHDRAWAL PERIOD	
	SCHEDULED	AS NEEDED	SCHEDULED	AS NEEDED	SCHEDULED	AS NEEDED

Peak flow (liters/min)						
Morning	415.9	424.1	414.4	424.5	414.8	427.3
Evening	436.3	441.1	441.3	445.2	433.6 P=0.005‡ P=0.021§	446.5
Peak-flow variability (%)¶	4.1	3.2	5.7 P<0.001‖	4.3	4.0 P<0.001‡	4.2
FEV_1 (liters)	3.09	3.13	3.04	3.12	3.06	3.12
Albuterol response (%)**	10.7	10.7	12.5 P=0.005††	9.2		
Extra albuterol (puffs/day)	1.4	1.6	1.3	1.6	1.6 P=0.013‡	1.6
Symptom score‡‡	0.4	0.4	0.4	0.4	0.4	0.4
Quality-of-life score‡‡	2.3	2.4	2.3	2.3	2.1 P=0.003‡ P=0.006§§	2.2 P=0.008 §§
PC_{20}(mg/ml)	0.73	0.73	0.56 p=0.013‖	0.72	0.66	0.76

*Values differ from those in Table 2 because Table 2 contains the mean data rather than estimates from the model.

†FEV_1 denotes forced expiratory volume in one second, and PC_{20} the concentration of methacholine required to decrease the FEV_1 by 20 percent.

‡P value is for the within-group comparison of the response at the end of the treatment period with that at the end of the withdrawal period.

§P value is for the comparison between groups of the change in response from the end of the treatment period to the end of the withdrawal period.

§Peak-flow variability was calculated as ([evening peak flow — morning peak flow] ÷ evening peak flow) x 100 (Martin et al., *Am. Rev. Respir. Dis.* 143:351, 1991).

‖P value is for the within-group comparison of the response at the end of the treatment period with that at the end of the run-in Period.

**Bronchodilator response was last measured during the run-in period at week 4 and during the active-treatment period at week 20.

††P value is for the comparison between groups of the change in response from the end of the run-in period to the end of treatment period.

‡‡See the footnotes to Table 2 for an explanation of the scoring system.

§§P value is for the within-group comparison of the response at the end of the run-in period with that at the end of the withdrawal period.

These results show that regular use of inhaled albuteral in patients with mild asthma is not generally associated with a deleterious effect on asthma control.

EXAMPLE 2

Correlation of β_2-Adrenergic Receptor Allele with Outcome of β-Agonist Administration

Materials and Methods:

GENERALLY: The patients analyzed in the present study had been participants in a β-agonist study, referred to as BAGS, sponsored by the Asthma Clinical Research Network of the United States National Heart, Lung and Blood Institute (ACRN). The results of that study are published in the *New England Journal of Medicine* (Drazen et al., *NEJM* 335:841, 1996, incorporated herein by reference); relevant portions of the Materials and Methods and Results sections of that paper are reproduced in Example 1. As noted above, that study concluded that, overall, no negative effects were associated with regular administration of albuterol to mild asthmatics.

We decided to further analyze the results of the BAGS trial by investigating the genotype of study participants' β_2-adrenergic receptor gene (encoding residue 16 of the protein). We were able to obtain materials for determining each patient's genotype for 179 of the 255 subjects. The remaining subjects either refused to participate or could not be located for genotyping.

ARMS ASSAY: The primers used to detect the β_2-adrenergic receptor gene polymorphism (corresponding to an A → G substitution at nucleotide 1633 of SEQ ID NO:2) (A → G) that gives rise to the Arg 16 → Gly amino acid change in the protein were: Wild-type forward primer A1 (5'-GCCTCTTGCTGGCACCCAA-<u>A</u>A-3' [SEQ ID

NO:3]) corresponding to nucleotides 1612-1633, except the penultimate base at the 3' end (underlined) was changed from T to A, polymorphism-specific forward primer A2 (5'-GCCTTCTTGCTGGCACCCAAAG-3' [SEQ ID NO:4], differs from the wild-type primer at the last nucleotide at 3' end (shown in bold), reverse primer Rev (5'-AGGATAACCTCATCCGTAAGG-3' [SEQ ID NO:5]) corresponding to nucleotides 2483-2503 on the complementary strand.

The primers used to detect the β_2-adrenergic receptor gene polymorphism (corresponding to a C → G substitution at nucleotide 1666 of SEQ ID NO:2) that gives rise to the Gln27 → Glu amino acid change in the protein were: Wild-type forward primer B1 (5'-CCGGACCACGACGTCACGCAAC-3' [SEQ ID NO:6] corresponding to nucleotides 1645-1666, except the penultimate base at the 3' end (underlined) was changed from G to A, polymorphism-specific forward primer B2 (5'-CCGGACCACGACGTCACGCAAG-3' [SEQ ID NO:7]), differs from the wild-type primer at the last nucleotide at 3' end (shown in bold), and reverse primer Rev.

Amplification by PCR of the genomic DNA of each sample included two reactions for each assay separately: one with wild type primers (A1 and REV) and the other with polymorphic (A2 and Rev) allele-specific primer set for polymorphism detection at nucleotide 16633 and wild type primers (B1 and Rev) and the polymorphic allele-specific primer set (B2 and Rev) for polymorphism detection at nucleotide 1666. Both polymorphism detection assays included human β-globin primer sets as positive controls in the PCR reaction mix. The primers for β-globin were: Forward primer BG1 (5'-GCTGTCATCACTTAGACCTC-3' [SEQ ID NO:8] corresponding to nucleotides 43-62 (Genbank accession no. L48217), reverse primer BG2 (5'-

CAGACGAATGATTGCATCAG-3' [SEQ ID NO: 9]) corresponding to nucleotides 766-785 on the complementary strand (Genbank accession no. L48217).

Each PCR reaction contained 5μl template DNA (buccal cell lysate or 100-200 ng of blood genomic DNA), PCR buffer II (Perkin Elmer), 1.5mM $MgCl_2$ (Perkin Elmer), 12.5 pmoles of each primer, 400 μM dNTPs (Perkin Elmer), 0.625 units of Taq polymerase (AmpliTaq polymerase, Perkin Elmer), 0.05 units of Perfect Match PCR enhancer (Stratagene) in a total volume of 25 μl. Conditions for PCR were: an initial hot start period of 5 min at 94°C, and temperature was hold at 80°C after the hot start during this period dNTPs and Taq polymerase were added. This was followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, 1 min at 72°C, with a final extension time of 5 min at 72°C. Thin walled 96 micro-well plates (Costar) were used with mineral oil for amplification reactions, in a PTC-100 thermal cycler (MJ Research, Watertown, MA). After amplification, about 20μl of reaction mixture was resolved by electrophoresis on a 2.0% agarose gel and stained with ethidium bromide for analysis.

Results

Figure 3 presents an example of the results we achieved in our genotype analysis of BAGS subjects. The overall findings for this residue are at β_2-adrenergic receptor residue 16 summarized below in Table 4:

TABLE 4
GENOTYPE OF BAGS STUDY PARTICIPANTS AT PROTEIN RESIDUE 16

RACE	AA	AG	GG	TOTAL
African American	7	12	11	30
Hispanic	2	9	3	14

Other Minority	1	4	4	9
Non-Minority	18	64	44	126
Total	28	89	62	179

These data indicate an allele frequency of 0.405 for Arg 16, so that one would expect approximately 16% of the population to be homozygous for this allele.

Having determined the β-adrenergic receptor genotype of BAGS participants, we then re-analyzed the BAGS data, stratifying it by the genotype of the individuals in the various treatment groups. Our results are shown in Figure 3. As can be seen, individuals who are homozygous for the Arg16 had an adverse response to the regular use of inhaled β-agonists, as indicated by a decrease in peak flow of 22.5 LPM between the end of the placebo "run-in" period and the end of the "withdrawal" period. By contrast, Arg16 homozygotes who received as-needed β-agonist treatment had a slight increase in peak flow over this interval. Gly16 homozygotes and Arg16/Gly16 heterozygotes treated with regularly-scheduled β-agonist administrations, did not display any adverse effects. These data indicate that the genotype at position 16 of the β_2-adrenergic receptor provides strong predictive information about the likely response of the patient to regularly-scheduled β-agonist treatment. The change in peak flow rate after regularly-scheduled albuterol treatment was significantly different over the interval between the end of the run period and the end of the withdrawal period in individuals homozygous for the Arg16 allele as compared with those having the Gly16/Gly16 or Arg16/Gly16 genotypes ($p=0.0019$ for AM PEFR; $p=0.0009$ for PM PEFR).

We note that all of the β-agonist-sensitive Arg16 homozygotes that we analyzed were also homozygous for Gln27, but other Gln27 homozygotes did not have adverse responses to β-agonists. Accordingly, we conclude that Gln27 is not likely to be an indicator, by itself, of susceptibility to adverse β-agonist responses.

EXAMPLE 3

Preferred Methods of Detecting β_2-Adrenergic Receptor Alleles

Generally:

As will be readily appreciated by those of ordinary skill in the art, any of a variety of techniques may be used to detect β_2-adrenergic receptor alleles in order to identify patients susceptible to a negative response to β-agonist administration in accordance with the present invention. The present Example is intended only to provide certain preferred examples of possible methods, and is not intended to limit the scope of the present invention.

Temperature Gradient Gel Electrophoresis:

GENERALLY: A temperature gradient gel electrophoresis method for detection of β_2-adrenergic receptor gene polymorphisms has been described (Reihsaus et al., *Am. J. Respir. Cell. Mol. Biol.* 8:334, 1993, incorporated herein by reference). Basically, a gradient of denaturing solvent in a polyacrylamide gel is employed to separate nucleic acid fragments that differ in sequence by only a single base pair (see, for example, Wartell et al., *Nuc. Acids Res.* 18:2699, 1990, incorporated herein by reference).

GEL ELECTROPHORESIS: Temperature-gradient gel electrophoresis can be carried out with a vertical acrylamide slab-gel apparatus, modified from a conventional vertical gel apparatus so that the glass plates containing the acrylamide gel are sandwiched between two aluminum heating blocks. Channels in the blocks allow circulating fluid to establish a temperature gradient from the top to bottom or from one side to the other. The channels running across the top and bottom are used to establish a temperature gradient in the same direction as electrophoretic migration. For a gradient perpendicular to DNA migration the fluid flows along the sides. Adhesive pipe tape is used to insulate the surfaces of the blocks not facing the glass. The rear block is placed against the main vertical support of the gel unit in the space formed by the overhanging upper buffer chamber. Both heating blocks rest on U-shaped plexiglass pieces which keep them above the buffer in the lower electrolyte chamber. Two thermostated fluid circulators (Haake Inc.) are employed to control the high and low temperatures.

The temperature gradient produced by the heating blocks is preferably checked for linearity and uniformity at least two temperature settings of the water circulators (e.g., 32°C/28°C and 44°C/18°C). A thermistor probe ($\pm$ 0.5°C) can be inserted into a gel to determine the gel temperatures at different depths and horizontal positions. For all temperature settings, the gradient in the gel is preferably linear and uniform within the region covered by the blocks. The appropriate percent acrylamide gel is determined according to standard practice. Such gels should be prepared in a denaturing solvent (e.g., 0.5M TBE, 7.0M urea, 40% formamide u/u) gels are loaded and according to standard procedures.

CALCULATION OF THERMAL STABILITY PROPERTIES: The model of the DNA helix-coil transmission can be used to calculate the thermal denaturation behavior of the DNA fragments (see Wartell et al., *Nuc. Acids Res.* 18:2699, 1990, and references cited therein). In addition to predicting the melting curve for a given DNA sequence, the calculation can also produce melting profiles for the base pairs in a DNA sequence. A melting profile displays the probability that the n^{th} base pair of the sequence is melted, $\theta(n)$, at a given temperature. From a three dimensional display of melting profiles at a series of temperatures the lengths and locations of cooperatively melting domains can be visualized. The calculation of the melting profiles assumes that strand dissociation is negligible.

The nearest neighbor stacking parameters can be obtained from McCampbell et al. (*Biopolymers* 28:1745, 1989), and Gotoh and Tagashira (*Biopolymers* 20:1033, 1981). All other parameters, such as the loop entropy factor, strand dissociation parameters, etc. can be obtained from McCampbell et al. (*Biopolymers* 28:1745, 1989). When only the first melting domain is of concern the dissociation parameters and loop entropy terms do not significantly influence theory-experiment comparisons. Extrapolations may be required to normalize the calculations to the solvent conditions utilized in the above-cited parameter references. For example, the Gotoh and Tagashira parameters, determined in 0.02M Na^+, can be extrapolated to 0.1 M Na^+ by scaling T_{AT} and T_{GC}, the average T_m's of AT and GC base pairs (Vologodskii et al., *J. Biomole Struct. Dynam.* 2:131, 1984).

Allele-Specific PCR:

GENERALLY: One preferred method for identifying β_2-adrenergic receptor gene polymorphisms in order to practice the present invention is to perform polymerase chain reactions (PCR) using primers whose 3'-most nucleotide is mismatched with respect to either the Arg 16 allele or the Gly 16 allele (see Newton et al., *Nuc. Acids. Res.* 17:2503, 1989, incorporated herein by reference; see also Example 2). The PCR reaction conditions are then adjusted so that product band is only produced when the primer and template are matched.

Useful PCR primers and conditions for detection of the Arg 16 and Gly 16 β2-adrenergic receptor gene alleles have been described (Turki et al., *J. Clin. Invest.* 95:1635, 1995, incorporated herein by reference; see also Example 2). As described in that article, allele-specific PCR is based on the premise that, under the appropriate conditions, a match between template and primer at the most 3' nucleotide is necessary for the generation of a PCR product (i.e., mismatches result in no product). Allele-specific PCR reactions can be performed, for example, as follows:

Genomic DNA is isolated, for example, from 2 ml of peripheral blood, by any available technique, such as the a cetylmethyl ammonium bromide separation technique (Jones et al., *Nature* 199:280, 1963). PCR reactions are carried out in a vol of 100 μl using ~ 500 ng of genomic DNA. Preferred primer pairs that delineate the two polymorphisms at nucleic acid 46 (amino acid 16), include i) 5'-CTTCTTGCTGGCACCCAAT<u>A</u>-3'(sense) (SEQ ID NO:10) and 5'-CCAATTTAGGAGGATGTAAACTTC-3' (antisense) (SEQ ID NO:11); ii) or the same antisense primer and 5'-CTTCTTGCTGGCACCCAAT<u>G</u>-3' (sense) (SEQ ID NO:12). The generated PCR product size using these primers is 913 bp. The polymerase Vent exo

(-) (New England Biolabs, Inc., Beverly, MA) can be used for these reactions. Reaction buffers are preferably those included with these polymerases from the manufacturers. Temperature cycling is preferably 98°C for 30 s, 66-68°C for 45 s, and 72°C for 45 s for 30 cycles, 20 μl of the PCR reactions can be electrophoresed on 1% agarose gels and visualized with ethidium bromide staining and ultraviolet illumination.

The allele-specific PCR technique can be verified by direct dideoxy sequencing of PCR products, preferably using sequencing primers different from those used in the PCR. In addition, plasmids consisting of wildtype β_2AR cDNA or mutated cDNA corresponding to the polymorphisms can be used as positive and negative control templates for the allele-specific PCR studies.

Hybridization Studies:

GENERALLY: It has long been appreciated that differences in nucleotide sequence can usually be detected by oligonucleotide hybridization under appropriate conditions. Recently, Hall et al. (*Lancet* 345:1213, 1995, incorporated herein by reference) have demonstrated that such techniques may reliably be used to detect sequence differences in the β_2-adrenergic receptor gene. As they teach, samples of genomic DNA containing the β_2-adrenergic receptor gene may be immobilized on a filter such as, for example, a Hybond® filter. In preferred methods, the relevant portion of the B_2-adrenergic receptor gene (i.e., a portion that includes nucleotide 46, encoding residue 16, is amplified by PCR, and the PCR product is affixed to the filter. The filter is then hybridized with excess unlabeled primer (to "block" nonspecific reactions). Subsequently, the filter is exposed to labeled primer under high-stringency conditions. The primer is designed to

hybridized with either the Gly16 or the Arg16 allele, but not with both under the hybridization conditions employed. In preferred embodiments, the filter is subsequently stripped and re-hybridized (or a duplicate filter is prepared and reacted in parallel) with a primer that reacts with the other allele.

Restriction Fragment Length Polymorphism:

The RFLP technique has long been a popular method for identifying sequence differences within a population (see, for example, Unit 2.7 of *Current Protocols in Human Genetics*, John Wiley & Sons, incorporated herein by reference). The β_2-adrenergic receptor gene contains a βan/site at position 523 (corresponding to amino acid 175 in the protein) that is polymorphic (see Ohe et al., *Tharax* 50:353, 1995). Although this polymorphism has not been shown to demonstrate linkage with the Arg16 → Gly polymorphism, standard techniques could readily be employed to detect such a linkage if it exists, so that utilized in the practice of the present invention.

Protein Assays

The presence of β_2-adrenergic receptor polymorphisms can also be detected through protein assays that can distinguish the Arg16 and Gly16 versions of the β_2-adrenergic receptor protein. For example, Western blots could be performed using monoclonal antibodies specific for either variant. Western blot technologies are well known in the art.

EXAMPLE 4

Kits for Identification of Individuals Susceptible to Adverse Responses to Chronic β-Agonist Therapy

As will be apparent to those of ordinary skill in the art, reagents useful in the practice of the present inventive methods can usefully be collected together in kits. For example, primer sets for allele-specific polymerase chain reaction studies can be provided together in a single container.

As described above in Example 3, β_2-adrenergic receptor gene alleles can be distinguished from one another through use of primers whose 3'-most nucleotides hybridize with one allele but are mismatched with respect to others. Examples 2 and 3 describe particular useful primer sets, but those of ordinary skill in the art will readily recognize that variations in precise primer sequence can be made without departing from the spirit or scope of the present invention, so long as one primer set produces an amplification product from one β_2-adrenergic receptor gene allele (e.g., the allele encoding the Arg16 variant), and a different primer set produces an amplification product from another allele (e.g., the allele encoding the Gly16 variant). Preferred allele-specific PCR kits also include other PCR reagents, such as buffer, salt solutions, dNTPs, control DNA including the Arg16 β_2-adrenergic receptor gene allele, control DNA including the Gly16 β_2-adrenergic receptor gene allele, and/or DNA polymerase. Preferably, the DNA polymerase is thermal-stable. Such kits may optionally include instructions for use.

Those of ordinary skill in the art will appreciate that analogous primer-containing kits may be prepared for ligation amplification reactions, which are based on the premise that adjacently-hybridized primers are only ligated together when their terminal residues are hybridized (see English et al., *Proc. Natl. Acad. Sci. USA* 9:630, 1994, incorporated

herein by reference). Additional reagents optionally included in ligantion amplification kits include buffers, salts, ligase (preferably thermal-stable ligase), ATP, control DNA including the Arg16 β_2-adrenergic receptor gene allele, control DNA including the Gly16 β_2-adrenergic receptor gene allele, and/or instructions for use.

Primer-containing kits may also be desirably prepared that do not contain allele-specific primer sets, but rather contain only a single set of primers, which primers amplify a region of the β_2-adrenergic receptor gene that encodes residue 16. Preferred such kits also include PCR reagents and/or sequencing reagents. Preferably, dideoxy sequencing reagents are employed (see Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, Chapter 13, incorporated herein by reference). Preferred dideoxy sequencing reagents include, for example, a sequencing primer (that hybridizes either to the β_2-adrenergic receptor gene amplification product or to a vector into which the product may be cloned), dNTPs, ddNTPs, buffers, salts, and/or instructions. In preferred embodiments, the dNTPs are provided either singly or in mixtures that are sets of three dNTPs. Preferred kits may also (or alternatively) include detection reagents, such as, for example, radioactive or fluorescent. Particularly preferred kits are designed genetic analyzers and include fluorescently-tagged primers.

Some preferred kits also contain reagents for distinguishing other β_2-adrenergic receptor gene alleles (e.g., alleles at other positions).

Other Embodiments

One of ordinary skill in the art will readily recognize that the foregoing has been merely a detailed description of certain preferred embodiments of the present invention. Various alterations and modifications of the procedures, techniques, and compositions described above will be apparent to those in the art and are intended to be encompassed by the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Drazen MD, Jeffrey

(ii) TITLE OF INVENTION: Diagnosing Asthma Patients Predisposed to Adverse Beta-Agonist Reactions

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Choate, HAll & Stewart
(B) STREET: 53 State Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Jarrell PhD, Brenda H.
(B) REGISTRATION NUMBER: 39,223
(C) REFERENCE/DOCKET NUMBER: 0092662-0010

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617 248 4000
(B) TELEFAX: 617 248 5000

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 413 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(vii) IMMEDIATE SOURCE:
(B) CLONE: Human Beta-2-Adrenergic Receptor Protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Gly Gln Pro Gly Asn Gly Ser Ala Phe Leu Leu Ala Pro Asn Arg
1               5                   10                  15

Ser His Ala Pro Asp His Asp Val Thr Gln Gln Arg Asp Glu Val Trp
            20                  25                  30
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Val Val Gly Met Gly Ile Val Met Ser Leu Ile Val Leu Ala Ile Val
        35                  40                  45

Phe Gly Asn Val Leu Val Ile Thr Ala Ile Ala Lys Phe Glu Arg Leu
    50                  55                  60

Gln Thr Val Thr Asn Tyr Phe Ile Thr Ser Leu Ala Cys Ala Asp Leu
65                  70                  75                  80

Val Met Gly Leu Ala Val Val Pro Phe Gly Ala Ala His Ile Leu Met
                85                  90                  95

Lys Met Trp Thr Phe Gly Asn Phe Trp Cys Glu Phe Trp Thr Ser Ile
            100                 105                 110

Asp Val Leu Cys Val Thr Ala Ser Ile Glu Thr Leu Cys Val Ile Ala
        115                 120                 125

Val Asp Arg Tyr Phe Ala Ile Thr Ser Pro Phe Lys Tyr Gln Ser Leu
    130                 135                 140

Leu Thr Lys Asn Lys Ala Arg Val Ile Ile Leu Met Val Trp Ile Val
145                 150                 155                 160

Ser Gly Leu Thr Ser Phe Leu Pro Ile Gln Met His Trp Tyr Arg Ala
                165                 170                 175

Thr His Gln Glu Ala Ile Asn Cys Tyr Ala Asn Glu Thr Cys Cys Asp
            180                 185                 190

Phe Phe Thr Asn Gln Ala Tyr Ala Ile Ala Ser Ser Ile Val Ser Phe
        195                 200                 205

Tyr Val Pro Leu Val Ile Met Val Phe Val Tyr Ser Arg Val Phe Gln
    210                 215                 220

Glu Ala Lys Arg Gln Leu Gln Lys Ile Asp Lys Ser Glu Gly Arg Phe
225                 230                 235                 240

His Val Gln Asn Leu Ser Gln Val Glu Gln Asp Gly Arg Thr Gly His
                245                 250                 255

Gly Leu Arg Arg Ser Ser Lys Phe Cys Leu Lys Glu His Lys Ala Leu
            260                 265                 270

Lys Thr Leu Gly Ile Ile Met Gly Thr Phe Thr Leu Cys Trp Leu Pro
        275                 280                 285

Phe Phe Ile Val Asn Ile Val His Val Ile Gln Asp Asn Leu Ile Arg
    290                 295                 300

Lys Glu Val Tyr Ile Leu Leu Asn Trp Ile Gly Tyr Val Asn Ser Gly
305                 310                 315                 320

Phe Asn Pro Leu Ile Tyr Cys Arg Ser Pro Asp Phe Arg Ile Ala Phe
                325                 330                 335

Gln Glu Leu Leu Cys Leu Arg Arg Ser Ser Leu Lys Ala Tyr Gly Asn
            340                 345                 350

Gly Tyr Ser Ser Asn Gly Asn Thr Gly Glu Gln Ser Gly Tyr His Val
        355                 360                 365

Glu Gln Glu Lys Glu Asn Lys Leu Leu Cys Glu Asp Leu Pro Gly Thr
    370                 375                 380
```

```
Glu Asp Phe Val Gly His Gln Gly Thr Val Pro Ser Asp Asn Ile Asp
385                 390                 395                 400

Ser Gln Gly Arg Asn Cys Ser Thr Asn Asp Ser Leu Leu
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3451 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(B) CLONE: HUman Beta-2-Adrenergic Receptor Gene

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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CCCGGGTTCA AGAGATTCTC CTGTCTCAGC CTCCCGAGTA GCTGGGACTA CAGGTACGTG     60
CCACCACACC TGGCTAATTT TTGTATTTTT AGTAGAGACA AGAGTTACAC CATATTGGCC    120
AGGATCTTTT GCTTTCTATA GCTTCAAAAT GTTCTTAATG TTAAGACATT CTTAATACTC    180
TGAACCATAT GAATTTGCCA TTTTGGTAAG TCACAGACGC CAGATGGTGG CAATTTCACA    240
TGGCACAACC CGAAAGATTA ACAAACTATC CAGCAGATGA AAGGATTTTT TTTAGTTTCA    300
TTGGGTTTAC TGAAGAAATT GTTTGAATTC TCATTGCATC TCCAGTTCAA CAGATAATGA    360
GTGAGTGATG CCACACTCTC AAGAGTTAAA AACAAAACAA CAAAAAAATT AAAACAAAAG    420
CACACAACTT TCTCTCTCTG TCCCAAAATA CATACTTGCA TACCCCCGCT CCAGATAAAA    480
TCCAAAGGGT AAAACTGTCT TCATGCCTGC AAATTCCTAA GGAGGGCACC TAAAGTACTT    540
GACAGCGAGT GTGCTGAGGA AATCGGCAGC TGTTGAAGTC ACCTCCTGTG CTCTTGCCAA    600
ATGTTTGAAA GGGAATACAC TGGGTTACCG GGTGTATGTT GGGAGGGGAG CATTATCAGT    660
GCTCGGGTGA GGCAAGTTCG GAGTACCCAG ATGGAGACAT CCGTGTCTGT GTCGCTCTGG    720
ATGCCTCCAA GCCAGCGTGT GTTTACTTTC TGTGTGTGTC ACCATGTCTT TGTGCTTCTG    780
GGTGCTTCTG TGTTTGTTTC TGGCCGCGTT TCTGTGTTGG ACAGGGGTGA CTTTGTGCCG    840
GATGGCTTCT GTGTGAGAGC GCGCGCGAGT GTGCATGTCG GTGAGCTGGG AGGGTGTGTC    900
TCAGTGTCTA TGGCTGTGGT TCGGTATAAG TCTGAGCATG TCTGCCAGGG TGTATTTGTG    960
CCTGTATGTG CGTGCCTCGG TGGGCACTCT CGTTTCCTTC CGAATGTGGG GCAGTGCCGG   1020
TGTGCTGCCC TCTGCCTTGA GACCTCAAGC CGCGCAGGCG CCCAGGGCAG GCAGGTAGCG   1080
GCCACAGAAG AGCCAAAAGC TCCCGGGTTG GCTGGTAAGG ACACCACCTC CAGCTTTAGC   1140
CCTCTGGGGC CAGCCAGGGT AGCCGGGAAG CAGTGGTGGC CCGCCCTCCA GGGAGCAGTT   1200
GGGCCCCGCC CGGGCCAGCC CCAGGAGAAG GAGGGCGAGG GGAGGGGAGG GAAAGGGGAG   1260
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GAGTGCCTCG CCCCTTCGCG GCTGCCGGCG TGCCATTGGC CGAAAGTTCC CGTACGTCAC 1320
GGCGAGGGCA GTTCCCCTAA AGTCCTGTGC ACATAACGGG CAGAACGCAC TGCGAAGCGG 1380
CTTCTTCAGA GCACGGGCTG GAACTGGCAG GCACCGCGAG CCCCTAGCAC CCGACAAGCT 1440
GAGTGTGCAG GACGAGTCCC CACCACACCC ACACCACAGC CGCTGAATGA GGCTTCCAGG 1500
CGTCCGCTCG CGGCCCGCAG AGCCCCGCCG TGGGTCCGCC CGCTGAGGCG CCCCCAGCCA 1560
NO: 2
GTGCGCTTAC CTGCCAGACT GCGCGCCATG GGGCAACCCG GGAACGGCAG CGCCTTCTTG 1620
N-4
GTGGCACCCA ATAGAAGCCA TGCGCCGGAC CACGACGTCA CGCAGCAAAG GGACGAGGTG 1680
NO:5 G
TGGGTGGTGG GCATGGGCAT CGTCATGTCT CTCATCGTCC TGGCCATCGT GTTTGGCAAT 1740
NO: 3 site
GTGCTGGTCA TCACAGCCAT TGCCAAGTTC GAGCGTCTGC AGACGGTCAC CAACTACTTC 1800
ATCACTTCAC TGGCCTGTGC TGATCTGGTC ATGGGCCTGG CAGTGGTGCC CTTTGGGGCC 1860
GCCCATATTC TTATGAAAAT GTGGACTTTT GGCAACTTCT GGTGCGAGTT TTGGACTTCC 1920
ATTGATGTGC TGTGCGTCAC GGCCAGCATT GAGACCCTGT GCGTGATCGC AGTGGATCGC 1980
TACTTTGCCA TTACTTCACC TTTCAAGTAC CAGAGCCTGC TGACCAAGAA TAAGGCCCGG 2040
GTGATCATTC TGATGGTGTG GATTGTGTCA GGCCTTACCT CCTTCTTGCC CATTCAGATG 2100
CACTGGTACC GGGCCACCCA CCAGGAAGCC ATCAACTGCT ATGCCAATGA GACCTGCTGT 2160
GACTTCTTCA CGAACCAAGC CTATGCCATT GCCTCTTCCA TCGTGTCCTT CTACGTTCCC 2220
CTGGTGATCA TGGTCTTCGT CTACTCCAGG GTCTTTCAGG AGGCCAAAAG GCAGCTCCAG 2280
AAGATTGACA AATCTGAGGG CCGCTTCCAT GTCCAGAACC TTAGCCAGGT GGAGCAGGAT 2340
GGGCGGACGG GGCATGGACT CCGCAGATCT TCCAAGTTCT GCTTGAAGGA GCACAAAGCC 2400
CTCAAGACGT TAGGCATCAT CATGGGCACT TTCACCCTCT GCTGGCTGCC CTTCTTCATC 2460
GTTAACATTG TGCATGTGAT CCAGGATAAC CTCATCCGTA AGGAAGTTTA CATCCTCCTA 2520
AATTGGATAG GCTATGTCAA TTCTGGTTTC AATCCCCTTA TCTACTGCCG GAGCCCAGAT 2580
TTCAGGATTG CCTTCCAGGA GCTTCTGTGC CTGCGCAGGT CTTCTTTGAA GGCCTATGGG 2640
AATGGCTACT CCAGCAACGG CAACACAGGG GAGCAGAGTG GATATCACGT GGAACAGGAG 2700
AAAGAAAATA AACTGCTGTG TGAAGACCTC CCAGGCACGG AAGACTTTGT GGGCCATCAA 2760
GGTACTGTGC CTAGCGATAA CATTGATTCA CAAGGGAGGA ATTGTAGTAC AAATGACTCA 2820
CTGCTGTAAA GCAGTTTTTC TACTTTTAAA GACCCCCCCC CCCCCAACAG AACACTAAAC 2880
AGACTATTTA ACTTGAGGGT AATAAACTTA GAATAAAATT GTAAAAATTG TATAGAGATA 2940
TGCAGAAGGA AGGGCATCCT TCTGCCTTTT TTATTTTTTT AAGCTGTAAA AAGAGAGAAA 3000
ACTTATTTGA GTGATTATTT GTTATTTGTA CAGTTCAGTT CCTCTTTGCA TGGAATTTGT 3060
AAGTTTATGT CTAAAGAGCT TTAGTCCTAG AGGACCTGAG TCTGCTATAT TTTCATGACT 3120
TTTCCATGTA TCTACCTCAC TATTCAAGTA TTAGGGGTAA TATATTGCTG CTGGTAATTT 3180
GTATCTGAAG GAGATTTTCC TTCCTACACC CTTGGACTTG AGGATTTTGA GTATCTCGGA 3240

CCTTTCAGCT GTGAACATGG ACTCTTCCCC CACTCCTCTT ATTTGCTCAC ACGGGGTATT 3300

TTAGGCAGGG ATTTGAGGAG CAGCTTCAGT TGTTTTCCCG AGCAAAGGTC TAAAGTTTAC 3360

AGTAAATAAA ATGTTTGACC ATGCCTTCAT TGCACCTGTT TGTCCAAAAC CCCTTGACTG 3420

GAGTGCTGTT GCCTCCCCCA CTGGAAACCG C 3451

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: wild-type forward primer A1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCCTCTTGCT GGCACCCAAA A 21

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: polymorphism-specific primer A2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCCTTCTTGC TGGCACCCAA AG 22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: reverse primer Rev

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGGATAACCT CATCCGTAAG G 21

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: wild-type forward primer B1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCGGACCACG ACGTCACGCA AC 22

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: polymorphism-specific formward primer B2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCGGACCACG ACGTCACGCA AG 22

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Beta-golbin forward primer BG1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCTGTCATCA CTTAGACCTC 20

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:
(B) CLONE: Beta-globin reverse primer BG2

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CAGACGAATG ATTGCATCAG 20

Claims

1. A method of identifying individuals susceptible to adverse responses to regular β-agonist administration, the method comprising steps of:

i) providing a genomic nucleic acid sample from an individual;

ii) identifying in said sample a first and second allele of the individuals β_2-adrenergic receptor gene; and

iii) classifying the individual as susceptible to adverse responses to regular β-agonist administration if the first and second alleles of the β_2-adrenergic receptor gene both encode Arg at residue 16 of the β_2-adrenergic receptor protein.

2. The method of claim 1 wherein the step of identifying employs a technique selected from the group consisting of: denaturing gel electrophoresis, allele-specific polymerase chain reaction amplification, single strand conformation polymorphism analysis, restriction fragment length polymorphism analysis, and allele-specific hybridization.

3. The method of claim 1, wherein the step of identifying comprises amplifying a first portion of the first β_2-adrenergic receptor gene allele, and a second portion of the second β_2-adrenergic receptor gene, which first and second portions each include a sequence encoding residue 16 of the β_2-adrenergic receptor protein.

4. The method of claim 3, wherein the step of identifying further comprises determining the nucleotide sequences of said portions.

5. The method of claim 4 wherein the step of determining constitutes automated sequence analysis.

6. The method of claim 3, wherein the step of identifying comprises amplifying said first portion through use of a primer set that amplifies a sequence encoding Arg at residue 16 of the β_2-adrenergic receptor protein but does not amplify a sequence encoding Gly at residue 16.

7. The method of claim 3, wherein the step of identifying comprises amplifying said first portion through use of a primer set that amplifies a sequence encoding Gly at residue 16 of the β_2-adrenergic receptor protein but does not amplify a sequence encoding Arg at residue 16.

8. The method of claim 6, wherein the primer set comprises a first primer having a nucleotide sequence including SEQ ID NO:3 and a second primer having a nucleotide sequence including SEQ ID NO:5.

9. The method of claim 7, wherein the primer set comprises a first primer having a nucleotide sequence including SEQ ID NO:4 and a second primer having a nucleotide sequence including SEQ ID NO:5.

10. A kit comprising:

a first set of primers selected to hybridize to a first portion of a human β_2-adrenergic receptor gene, which first portion includes a sequence encoding position 16 of said human β_2-adrenergic receptor, in such a manner that, when used in a polymerase chain reaction, said second set of primers amplifies said portion when position 16 is Arg but not when position 16 is Gly; and

a second set of primers selected to hybridize to said first portion of a human β_2-adrenergic receptor gene in such a manner that, when used in a polymerase chain reaction, said second set of primers amplifies said portion when position 16 is Gly but not when position 16 is Arg,

set first and second sets of primers being provided together in a container.

11. The kit of claim 10 further comprising a component selected from the group consisting of: amplification buffer, water, DNA polymerase, first control DNA including a first human β_2-adrenergic receptor gene that encodes Arg at human β_2-adrenergic receptor position 16, second control DNA including a second human β_2-adrenergic receptor gene allele that encodes Gly at human β_2-adrenergic receptor position 16, instructions for use, and combinations thereof.

12. A kit comprising:

a primer set selected to hybridize to a human β_2-adrenergic receptor gene in which a manner that, when used in a polymerase chain reaction, the primer set amplifies a portion of said human β_2-adrenergic receptor gene, which portion includes a sequence encoding human β_2-adrenergic receptor residue 16; and

reagents for determining the nucleotide sequence of said amplified portion, said primer set and reagents being arranged together in a container.

13. The kit of claim 12, wherein said reagents are selected from the group consisting of: a sequencing primer that hybridizes to a piece of said amplified portion in such a way that allows extension across said sequence encoding human β_2-adrenergic receptor residue 16, DNA polymerase, dNTPS, ddNTPs, buffer, and combinations thereof.

14. The kit of claim 12, wherein said sequencing primer is fluorescently labeled for use in an automated genetic analyzer.

15. The kit of claim 12 further comprising a component selected from the group consisting of amplification buffer, water, DNA polymerase, first control DNA including a first human β_2-adrenergic receptor gene that encodes Arg at human β_2-adrenergic receptor position 16, second control DNA including a second human β_2-adrenergic receptor gene allele that encodes Gly at human β_2-adrenergic receptor position 16, instructions for use, and combinations thereof.

16. A kit comprising:

an oligonucleotide primer that hybridizes to a portion of a human β_2-adrenergic receptor gene, which portion includes a sequence encoding residue 16 of the human β_2-adrenergic receptor, said oligonucleotide having higher affinity for said portion when said sequence encodes Arg than it has when said sequence encodes Gly; and

an oligonucleotide primer that hybridizes to a portion of a human β_2-adrenergic receptor gene, which portion includes a sequence encoding residue 16 of the human β_2-adrenergic receptor, said oligonucleotide having higher affinity for said portion when said sequence encodes Gly than it has when said sequence encodes Arg.

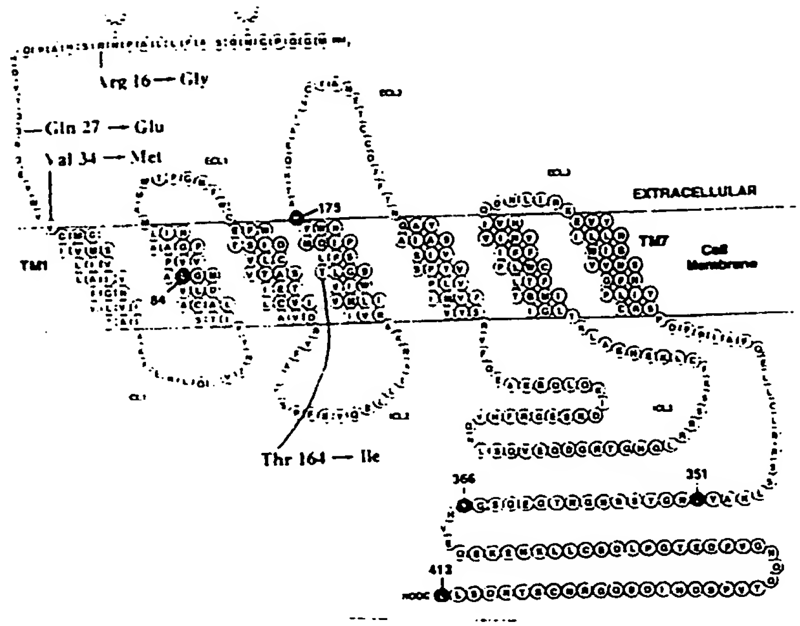
Arg 16 — Gly
Gln 27 — Glu
Val 34 — Met
EXTRACELLULAR
175
TM1
84
TM7
Cell Membrane
Thr 164 — Ile
366
351
413
HOOC

Figure 1

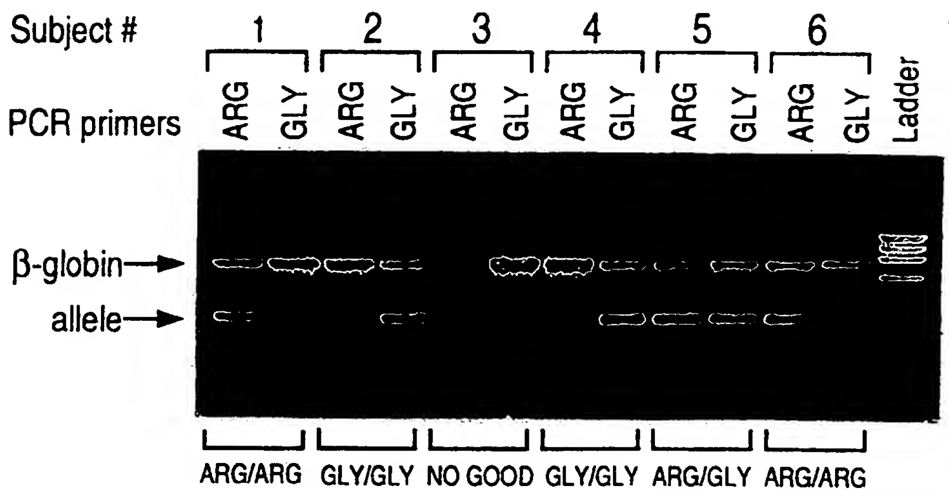
Subject #
1
2
3
4
5
6
PCR primers
ARG
GLY
ARG
GLY
ARG
GLY
ARG
GLY
ARG
GLY
ARG
GLY
Ladder
β-globin
allele
ARG/ARG
GLY/GLY
NO GOOD
GLY/GLY
ARG/GLY
ARG/ARG

Figure 2

Figure 3A.

Figure 3B.